with a wide range of applications in biological research and molecular therapeutics. Zinc finger proteins (ZFPs), belonging to Cys2His2 family constitute the most common DNA binding motifs found in eukaryotes. ZFP normally occur in repeats of two to three zinc finger motifs (ZFMs) to bind 6-9 contiguous DNA base pairs in a sequence specific manner. Several methods of varying complexity are available to engineer ZFPs that can target all the 64 codons in the genome. Although ZFPs are becoming a powerful tool for site specific modification in the genome, several challenges remain before the full potential of ZFPs can be realized. The engineered ZFPs generated using the present design platforms target mostly base triplets with 5' Guanine (GNN, where N is any nucleotide) and the non-GNN or AT rich modules are difficult to target. In the present project we attempt to address this challenge by designing linker regions between the ZFP motifs to target non-contiguous base pairs in the DNA. This will increase the number of targetable DNA sequences by an order of magnitude and will help to realize the full potential of ZFPs. Using structure based methods, we provide an extensive library of possible linker molecules that can be introduced between the individual zinc finger motifs to skip up to 10 base pairs between adjacent zinc finger protein recognition sites in the DNA sequences. We also performed a proof of principle experiment to validate the binding affinity and specificity of one of the computationally designed ZFP to its target DNA sequence.

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The Increased Affinity and Decreased Selectivity of the HPV6 E2ΔLL Mutant Stems from a Decrease in DNA Bending in the Mutant Complex

Geoffrey M. Gray, Arjan van, der Vaart.
Department of Chemistry, University of South Florida, Tampa, FL, USA.

The human papillomavirus type 6 E2 protein binds the 5'-AACCG-NNNN-CGGTT-3' DNA consensus sequence, where NNNN is a linker region. The wild-type (WT) protein only binds A/T rich linker regions in a binding process kinetically characterized by a fast phase followed by a slow phase, whereas the E2ΔLL mutant shows enhanced affinity for both A/T and G/C rich linker sequences with a significantly sped up fast phase. To rationalize these observations, we performed long molecular dynamics simulations of the WT bound to DNA with AATT and CCGG linkers, and of E2ΔLL bound to the same sequences. It was found that the DNA bending angle was decreased to 25° for both E2ΔLL-DNA complexes, while the bending angle was 60° in the WT complexes. The decreased bending angle lowers the DNA distortion energy in the mutant complexes, explaining the increased binding affinity of the E2ΔLL protein. Moreover, the reduction in bending energy increases the population of pre-bent structures, which explains the enhanced fast phase of binding in the mutant. In contrast to earlier hypotheses which focused on increased protein flexibility, our simulations show that the increased binding affinity and decreased selectivity of the mutant stems from a decrease in DNA bending.

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Single-Molecule View on the Duality of Microrna Uridylation

Luuk Loeff[1], Minju Ha[2], Boseon Kim[2], Kyu-Hyeon Yeom[1,2], Dhirendra K. Simanshu[3], Dinshaw J. Patel[3], Narry V. Kim[2], Chirlmin Joo[1].
[1]Department of BioNanoScience, Kavli Institute of NanoScience,Technical University Delft, Delft, Netherlands, [2]Institute for Basic Science and School of Biological Sciences, Seoul National University, Seoul, Korea, Republic of, [3]Structural Biology Program, Memorial Sloan-Kettering Cancer Centre, New York, NY, USA.

Terminal uridylyl transferases are a class of enzymes that play a key role in both biogenesis and degradation of microRNAs (miRNAs) in eukaryotes. Previously, we and other groups showed that TUT4 uridylates precursor microRNA (pre-miRNA) in coordination with Lin28 and thus acts as a post-transcriptional repressor of microRNA maturation. Using single-molecule FRET, we show that TUT4 maintains the tight contact with pre-miRNA and Lin28 while it captures the 3' end of RNA and brings this to its catalytic domain. This mechanism leads to the formation of a unique closed loop of the U tail. Besides this repression pathway, terminal uridylyl transferases are also able to enhance miRNA biogenesis through mono-uridylation of pre-miRNA substrates when Lin28 is absent. Using single-molecule fluorescence spectroscopy, we show that TUT7 exhibits preference for group II pre-miRNAs by regulating their binding frequency. In conclusion, our study provides insight into the duality of miRNA uridylation and may give a hint to a general mechanism of action of terminal uridylyl transferases.

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Single Molecule DNA Stretching Studies of DNA Intercalation

Ioulia Rouzina.
BMBB, University of Minnesota, Minneapolis, MN, USA.

Many proteins and ligands bind double stranded (ds) DNA by inserting their hydrophobic residues in between the DNA bases, either partially or completely, thereby locally elongating, bending and unwinding the dsDNA. This intercalative binding mode is typical of many proteins that control transcription by locally deforming the dsDNA, such as HMG-type proteins or TATA box binding proteins. Also intercalative binding is typical of many small aromatic molecules that are used either as the research tool for dsDNA imaging (EtBr or Yo-Yo), or as anti-cancer drugs (ActinomysinD or Ruthenium "threading" intercalators). Conventional approaches to the study of intercalative binding are often limited as many of these molecules also have the non-intercalative binding modes, may cause DNA aggregation, intercalate weakly, or too slowly. Recently the single molecule stretching of polymeric dsDNA with Optical Tweezers in the presence of several intercalating molecules was employed to characterize their equilibrium dissociation constant, DNA elongation and the binding site size. In this theoretical work we discuss how the complete stretching curves of the DNA-intercalator complexes can be used to study the proteins with weak intercalative ability and slow binding kinetics, the effect of intercalators on the dsDNA duplex stability, flexibility and elasticity. In some cases, even the protein-induced dsDNA bending angle and elongation can be quantified based on the intercalator - dsDNA titrations coupled to DNA stretching. This approach also offers a new way to study the contributions of individual intercalating and non-intercalating groups of the protein to its DNA intercalative ability. For some proteins, such as nucleocapsid proteins of retroviruses, discovery of their intercalative nature by DNA stretching may suggest their novel physiological roles.

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Nucleic Acid Binding Kinetics of HIV-1 Nucleocapsid Proteins from Single Molecule DNA Stretching

Jialin Li[1], Robert Gorelick[2], Ioulia Rouzina[3], Williams Mark[1].
[1]Northeastern University, Boston, MA, USA, [2]AIDS and Cancer Virus Program, Leidos Biomedical Research, Inc., Frederick, MD, USA, [3]University of Minnesota, Minneapolis, MN, USA.

The human immunodeficiency virus type 1 (HIV-1) Gag protein is essential for retroviral assembly. During viral maturation, Gag is processed to form matrix (MA), capsid (CA), and nucleocapsid (NC) proteins. Mature NCp7 is derived fromprocessing of NCp15 and NCp9. NCp7 functions as a nucleic acid chaperone during retroviral replication, in which it rearranges nucleic acids to facilitate reverse transcription and recombination. In this work, we use single-molecule DNA stretching to probethe interactions of these proteins with DNA. We find that NCp7 intercalates into double-stranded DNA to keep the two single strands close together while destabilizing them. Surprisingly, multiple stretch and release cycles of DNA in the presence of NCp7 yield changing force-extension curves on the time scale of tens of minutes. If the NCp7 solution is rinsed from the buffer surrounding the DNA molecule, we find that some fraction of the bound protein does not dissociate. The protein only dissociates completely when competitor DNA is introduced to the solution surrounding the stretched DNA molecule. Thus, NCp7 exhibits binding modes on multiple time scales, including both rapid microscopic and slow macroscopic dissociation rates, which is also examined through pulling rate dependence studies. To test the origin of this behavior and understand how NC-DNA interactions are regulated, we also apply these methods to NCp9 and NCp15 interactions with DNA.

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Partial Unwrapping of SSB from SsDNA Facilitates RecA Filament Formation

Sukrit Suksombat[1], Alexander G. Kozlov[2], Timothy M. Lohman[2], Yann R. Chemla[1,3].
[1]Department of Physics, University of Illinois at Urbana-Champaign, Urbana, IL, USA, [2]Department of Biochemistry and Molecular Biophysics, Washington University School of Medicine, St. Louis, MO, USA, [3]Center for the Physics of Living Cells, Center for Biophysics and Computational Biology, Champaign-Urbana, IL, USA.

DNA breaks can be repaired by homologous recombination, a process that maintains genetic stability in an organism. A protein that is essential for this mechanism in E. coli is RecA. During repair, RecA must bind and form nucleoprotein filaments on single-stranded DNA (ssDNA) in direct competition with single-stranded DNA binding protein (SSB). Despite extensive studies, the mechanism behind this competitive process remains unclear.